

LC-MS/MS Analysis of 16 PFAS in Salmon using QuEChERS based on FDA Method C-010.02

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Introduction

Per- and polyfluorinated alkyl substances (PFAS) are a class of compounds which have been widely used as commercial product applications over the past decades due to their versatile physical and chemical properties (e.g., water repellent, firefighting foams, cookware, food packaging). As a result of their chemical stability, these compounds are also widely present in our environment and have the potential risk of bioaccumulation in human over time. To avoid possible human health risks (e.g., low infant birth weights, cancer, effects on the immune system), for certain substances limit values and analytical methods were introduced by regulatory agencies (e.g., EPA, FDA).¹⁻⁴

The U.S. Food and Drug Administration (FDA) issued advisories for PFAS extraction from food samples applying modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) methodology extraction and clean-up step (using dispersive solid phase extraction - dSPE), and an additional clean-up of the obtained QuEChERS extract using weak anion exchange (WAX) SPE columns, followed by LC-MS/MS analysis.⁴

For FDA method C-010.02⁴, an extraction salt mixture containing 6.0 g MgSO₄ as well as 1.5 g NaCl, and a dSPE clean-up mix containing 900 mg MgSO₄, 300 mg primary secondary amine (PSA) and 150 mg graphitized carbon black, are specified. The Supel[™] QuE non-buffered extraction salt mix (**55295-U**) and the newly developed Supel[™] QuE PSA/ENVI-Carb[™] Tube 3 for clean-up (**55479-U**) meet these method

requirements. For further clean-up in this study, Supelclean[™] ENVI-WAX[™] SPE cartridges were used as a direct equivalent to the specified SPE cartridges in the FDA method C-010.02.

This application note describes the analysis of 16 PFAS compounds in salmon using LC-MS/MS and was performed in accordance with FDA method C-010.02.

Experimental

Solutions and Standards Preparation

Native and isotopically labeled PFAS standards were obtained as methanolic 50 µg/mL stock solutions. These standards were then diluted with methanol following the dilution scheme of the method C-010.02 to obtain the calibration standards in the required concentration range (external calibration: 0.01, 0.05, 0.10, 0.50, 1.0, 5.0, 10, and 25 ng/mL).

Sample Preparation

In accordance with U.S. FDA method C-010.02, analysis was performed for salmon samples. The salmon samples (5 g, homogenized with knife mill) were spiked at 0.05, 0.5 or 2.0 ng/g with 16 native PFAS as well as further spiked with 8 isotopically labeled internal standards at 2 ng/g. After adding 5 mL water, 150 µL formic acid and 10 mL acetonitrile, the samples were vigorously shaken for PFAS extraction. To test for potential PFAS background contamination, salmon samples were only spiked with isotopically

labeled internal standards and equally processed. After addition of the Supel™ QuE salt package (6.0 g MgSO₄, 1.5 g NaCl, **55295-U**), the mixture was placed on a shaker for 10 minutes at 1500 rpm, followed by centrifugation for 10 minutes at 4000 g. The obtained supernatant was transferred into a second tube, the Supel™ QuE PSA/ENVI-Carb™ Tube 3 (contains 900 mg MgSO₄, 300 mg PSA, 150 mg ENVI-Carb™, **55479-U**), and the sample was shaken for 10 minutes at 1500 rpm, followed by centrifugation for 10 minutes at 4000 g. Subsequently, the supernatants were filtered using Millex® Nylon 0.2 µm syringe filters (**SLGNX13**).

For salmon samples, further SPE clean-up is required prior to LC-MS/MS analysis in accordance with the above FDA method. Therefore, 1 mL of the filtered supernatant was transferred into a tube and diluted to 15 mL with water. Supelclean™ ENVI-WAX™ SPE cartridges were used (500 mg, 6 mL, **54057-U**) for the SPE clean-up, conditioned with 9 mL of 0.3% NH₄OH in MeOH and equilibrated with 5 mL of water. After the salmon sample (15 mL) was loaded and passed through the cartridge, 5 mL of water was added as a washing step. The cartridge was subsequently dried for 1 min before 4 mL 0.3% NH₄OH in MeOH was used to elute the analytes into an additional tube. Finally, the sample was evaporated to dryness, reconstituted in 1 mL MeOH and 5 µL of the internal standard N-EtFOSAA-d₅ (200 ng/ml) were added prior to LC-MS/MS analysis.

LC-MS/MS analysis

An Agilent 1290 Infinity II instrument coupled to an Agilent 6495C triple quadrupole mass spectrometer was used for the LC-MS/MS analysis. Analyte separation was achieved using a Purospher® Star RP-18 endcapped Hibar HR (15 cm x 2.1 mm, 2 µm, **1.50649**) as analytical column. In addition, a delay column (Purospher® Star RP-18 endcapped Hibar RT (5 cm x 4.0 mm, 3 µm, **1.50428**) was positioned after the mixing valve and before the autosampler to offset potential PFAS contamination originating from the LC system (e.g., pump, tubings, fittings, filters). The Purospher® STAR HPLC columns were chosen, as they generally provide stable HPLC separations even at higher pH, e.g. in the presence of basic mobile phase additives, such as 1-methyl piperidine recommended to be used in the C-010.02 method. We have tested the method with and without addition of the 1-methyl piperidine to the mobile phase. As no significant sensitivity gain was obtained using the additive in the presented application, however, the separation was performed without using 1-methyl piperidine, but the column was kept for this application to be prepared also for basic MP conditions. In order to avoid possible PFAS adsorption to the glass surface, polypropylene snap caps vials were used instead of standard glass vials. The LC and MS conditions are listed in **Table 1**.

Table 1. LC conditions used for PFAS analysis of 16 compounds

LC Conditions			
Instrument:	Agilent 1290 Infinity II instrument coupled to an Agilent 6495C triple quadrupole mass spectrometer		
Columns:	Purospher® STAR RP-18 endcapped, Hibar HR, 2.0 µm, 15 cm x 2.1 mm (1.50649) Delay Column: Purospher® Star RP-18 endcapped, Hibar RT, 3.0 µm, 5 cm x 4.0 mm (1.50428)		
Mobile phase:	[A] 5 mM Ammonium acetate*; [B] methanol		
	Time (min)	A%	B%
	Initial	90.0	10.0
	3.0	90.0	10.0
Gradient:	3.1	60.0	40.0
	26.0	10.0	90.0
	26.1	90.0	10.0
	28.0	90.0	10.0
Flow rate:	0.30 mL/min		
Column temp.:	40 °C		
Detector:	MS (ESI-), MRM (see Table 2 for details)		
Injection volume:	5 µL		

* Mobile phase A was adjusted compared to FDA method C-010.02 and used without addition of 1-methyl piperidine

Table 2. MRM, chromatographic and linearity (R²) data for analysis of the 16 PFAS

Peak	Acronym	Compound	MRM	Collision energy	RT (min)	R ²
1	PFBA	Perfluorobutanoic acid	213.0→169.0	4	7.2	0.9969
2	PFPeA	Perfluoropentanoic acid	263.0→219.0	4	10.0	0.9972
3	PFBS	Perfluorobutanesulfonic acid	298.9→80.0	40	10.6	0.9967
4	PFHxA	Perfluorohexanoic acid	313.0→269.0	4	12.9	0.9969
5	PFPeS	Perfluoropentanesulfonic acid	348.9→99.0	37	13.3	0.9962
6	HFPO-DA	Hexafluoropropylene oxide dimer acid	285.0→169.0	4	13.6	0.9969
7	PFHpA	Perfluoroheptanoic acid	363.0→319.0	4	15.5	0.9924
8	PFHxS	Perfluorohexanesulfonic acid	398.9→99.0	41	15.8	0.9935
9	NaDONA	Sodium dodecafluoro-3H-4,8-dioxananoate	377.0→251.0	8	15.8	0.9985
10	PFOA	Perfluorootanoic acid	413.0→369.0	8	17.8	0.9988
11	PFHpS	Perfluoroheptanesulfonic acid	448.9→99.0	45	17.9	0.9969
12	PFNA	Perfluoronanoic acid	463.0→419.0	8	19.8	0.9973
13	PFOS	Perfluorooctanesulfonic acid	498.9→80.0	76	19.8	0.9957
14	9Cl-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	530.9→351.0	28	20.7	0.9899
15	PFDA	Perfluorodecanoic acid	513.0→469.0	8	21.4	0.9822
16	11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	630.9→451.0	32	23.5	0.9978

Results and Discussion

A chromatogram of a solvent calibration standard containing the 16 native compounds is shown in **Figure 1**. The lower limit of quantitation (LLOQ) for all 16 compounds was 0.01 ng/mL for the LC-MS/MS method, and 0.02 ng/g in relation to the salmon sample. Linear calibration curves (0.01 –25 ng/mL) with R² ≥0.99 were obtained for all PFAS analytes except for PFDA with 0.98 (**Table 2**).

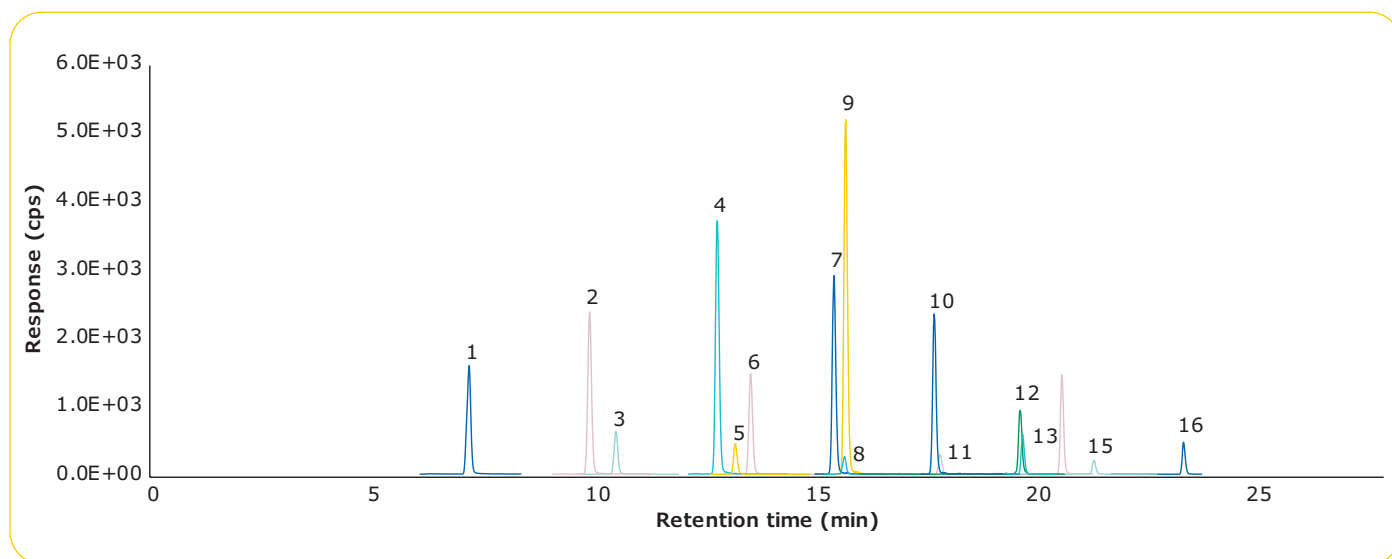


Figure 1. 16 PFAS compounds at 1 ng/mL in methanol (Peak ID see **Table 2**)

The acceptable recovery range for the investigated PFAS analytes based on the FDA guidelines for the validation of chemical methods in food, feed, cosmetics and veterinary products is 40-120% (including RSD \leq 22%) for concentrations at 0.001 mg/kg (1 ng/g). **Table 3** displays the recoveries and %RSD from the experimental study where 16 compounds were spiked in triplicate to salmon samples at concentration levels of 0.05, 0.5, and 2.0 ng/g. The background evaluation of PFAS in the used salmon (salmon sample only spiked with isotopically labeled internal standards) revealed analyte concentrations for 3 PFAS analytes present above LLOQ (0.02 ng/g for PFBA and PFOS, 0.04 ng/g for PFOA). To accurately calculate the analyte recovery at the lowest spiking level (0.05 ng/g) for PFBA, PFOS and PFOA, the background levels found in salmon were subtracted accordingly. All obtained recoveries and %RSD were in the recommended range and thus meet the requirements of the FDA method.⁵

Table 3. Precision and recovery (n = 3) of PFAS in salmon samples at 3 fortification levels (0.05 ng/g, 0.5 ng/g and 2.0 ng/g)

Analyte	Fortified conc. 0.05 ng/g		Fortified conc. 0.5 ng/g		Fortified conc. 2.0 ng/g	
	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
PFBA	88.6**	1.4	81.5	2.1	71.5	0.9
PFPeA	98.8	8.1	76.9	3.1	71.4	1.6
PFBS	97.6	1.6	77.7	3.0	71.8	3.1
PFHxA	107.3	2.0	77.6	3.1	71.2	2.2
PFPeS	97.9	3.4	80.5	3.3	69.6	4.5
HFPO-DA	92.5	3.7	75.7	4.0	72.2	1.8
PFHpA	111.7	9.6	79.2	7.7	70.1	2.5
PFHxS	104.0	10.9	85.6	4.0	75.1	3.9
NaDONA	92.6	3.4	72.8	1.9	70.2	0.2
PFOA	91.8 **	14.1	97.9	6.0	78.4	0.4
PFHpS	86.9	13.8	75.8	4.3	66.3	3.5
PFNA	99.9	13.9	96.4 (87.0 & 105.7) *	N/A	89.8	21.9
PFOS	88.4** (85.2&91.5) *	N/A	86.4	2.2	75.0	8.0
9CI-PF3ONS	82.3	7.7	70.8	4.4	64.6	2.2
PFDA	82.6 (71.2 & 94.0) *	N/A	78.0 (73.1 & 82.8) *	N/A	82.6 (70.9 & 94.3) *	N/A
11CI-PF3OUdS	90.7	6.6	63.0	4.1	56.0	2.1

* n = 2, due to discarding of outlier

** Analyte recovery at lowest spiking level obtained after subtraction of background level in salmon (0.02 ng/g for PFBA and PFOS, 0.04 ng/g for PFOA)

Conclusions

In this application note, the workflow of the FDA method C-010.02 for analysis of 16 PFAS in salmon samples based on a QuEChERS approach was investigated. At fortification levels of 0.05 ng/g, 0.5 ng/g and 2.0 ng/g, recoveries for all 16 compounds were within the acceptable range specified in the relevant FDA guideline for method validation. The calculated %RSDs were below 22%, further indicating a satisfactory precision. Hence, the used new Supel™ QuE PSA/ENVI-Carb™ Tube 3 clean-up mix (**55479-U**), in combination with the Supel™ QuE extraction salt mix, the Supelclean™ ENVI-WAX™ SPE cartridges, and the Millex syringe filters proved to be suitable sample preparation products to achieve desired cleanliness during PFAS analysis in food samples. The Purospher® STAR RP-18 HPLC columns were used in this method, as these generally provide stable separations in a wider range of mobile phase pH values, e.g. in the presence of basic mobile phases additives, such as 1-methyl piperidine described in the C-010.02 method.

Featured & Related Products

Description	Cat. No.
Sample Prep	
Supel™ QuE PSA/ENVI-Carb™ Tube 3 volume 15mL, Pk.50	55479-U
Supel™ QuE Non-Buffered Tube 2, pk. 50	55295-U
Supelclean™ ENVI-WAX™ SPE Tube, 500 mg, volume 6 mL, Pk.30	54057-U
Visiprep™ SPE Manifold standard, 12-port model	57030-U
Millex® Syringe Filter, Nylon, Non-sterile, 0.20 µm pore size, 13 mm diameter	SLGNX13
HPLC	
Purospher® STAR RP-18 endcapped Hibar® HR, 2 µm, 15 cm x 2.1 mm	1.50649
Purospher® STAR RP-18 endcapped Hibar® RT, 3 µm, 5 cm x 4.0 mm	1.50428
Solvents & Reagents	
Acetonitrile hypergrade for LC-MS LiChrosolv®	1.00029
Methanol hypergrade for LC-MS LiChrosolv®	1.06035
Water for UHPLC-MS LiChrosolv®	1.03728
Ammonium acetate LiChropur™, eluent additive for LC-MS	73594
Formic acid for LC-MS LiChropur™	5.33002
Ammonium hydroxide for HPLC LiChropur™	5.43830
Standards	
Perfluorobutanoic acid, analytical standard, 25 mg	68808
Perfluoropentanoic acid, analytical standard, 25 mg	68542
Perfluorohexanoic acid, analytical standard, 25 mg	43809
Perfluoroheptanoic acid, analytical standard, 25 mg	43996
Perfluorooctanoic acid, analytical standard, 100 mg	33824
Perfluorodecanoic acid, analytical standard, 25 mg	43929
Pentadecafluorooctanoic acid, 100 µg/mL in methanol, analytical standard, 1 mL	33603
Heptadecafluorooctanesulfonic acid, 100 µg/mL in methanol, analytical standard, 1 mL	33607
Accessories	
Large Volume SPE Reservoir, volume 25 mL, Pk. 30	54258-U
BRAND® PP graduated centrifuge tube, screw cap, volume 50 mL, without base, non-sterile, Pk. 300	BR114820
BRAND® PP graduated centrifuge tube, screw cap, volume 15 mL, without base, non-sterile, Pk. 750	BR114817
Syringe PP/PE without needle, 10 mL	Z683590

Read more about PFAS testing at

[SigmaAldrich.com/pfas](https://www.sigmaaldrich.com/pfas)

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3. Draft Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/ MS. United States Environmental Protection Agency, Office of Water, August 2021. https://www.epa.gov/system/files/documents/2021-09/method_1633_draft_aug-2021.pdf
4. Method C-010.02 Determination of 16 Per and Polyfluoroalkyl Substances (PFAS) in Processed Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). United States Food and Drug Administration, December 2021. <https://www.fda.gov/media/131510/download>
5. Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products. United States Food and Drug Administration, October 2019. <https://www.fda.gov/media/81810/download>

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